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In-vitro Anti-Oxidant and Anti-Diabetic Activity of Ethanolic Extract of *Mangifera Indica Leaves*

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ABSTRACT
Mango, which is also popularly known as king of fruits, botanically called as Mangifera indica which as multiple benefits for the whole plant according to traditional knowledge as well as literature search. The present study is aimed to prepare ethanolic extract of the shade dried leaves and it should be subjected for preliminary phytochemical screening to know about the secondary metabolites present in the extract and further subjected for determination of Total Phenolic and Total Flavonoid and In-vitro anti-oxidant activity using DPPH scavenging assay. The present study reveals that ethanolic extract of Mangifera indica shows potent inhibition activity at different concentrations when compared with standard ascorbic acid and it has showed significant effect at 60µg/ml of extract with 10µg/ml. It is reveal that at lower concentration having high amount of phenols and flavonoids are inhibit effects is considered for the potent for further studies.

Keywords
Mangifera indica,
Ethanol, Phenol,
Flavonoid, DPPH assay

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Introduction

The medicinal plants are considered as a rich source of ingredients which can be used in drug development and synthesis. Besides that, these plants play a critical role in the development of human culture around the world. Moreover, some plants are considered as an important source of nutrition and as a result these plants are recommended for their therapeutic values. It has been estimated that about 13,000 species of plants have been employed for at least a century as traditional medicines by various cultures around the world. A list of over 20,000 medicinal plants has been published, and very likely a much larger number of plants [(1).]

Plant profile

Taxonomical Classification

Kingdom: plantae, order: spindles, Family: Anacardiaceous, genus: Mangifera, species: indica.

Synonyms

Greek: manko, mango, **English**: mango, **Hindi**: am (or**)** ambi, **Telugu**: mamudi, **Tamil**: mampalam.

Fig.1: whole plant of mango tree

Mangifera indica is a large evergreen tree, with a dome -shaped crown. It belongs to the family anacardiaceous. It is found all over the tropical regions of the world. It is used as a medicinal plant [(2)]. Mangifera indica leaves are alternative, simple, lethargy, oblong-lanceolate, curved upwards from the midrib and sometimes with edges a little wavy [(3)]. Mango stomata are anomocytic types which are bordered by a varying number of cells and not different from the epidermis, they are small in size and presented only on the lower surface of the leaf [(4)].

Chemical Constituents of Mangifera indica mainly consists Mangifera, phenolic compounds, benzophenones, flavonoids, carotenoids, quercetin, isoquercetin, ascorbic acid, and tocopherols. Terpenoids, carbohydrates, sterols, carotenoids, vitamins, fatty acids, amino acids [(5)]. Mangiferin is the main contributor of most of the biological activities of Mangifera indica's extract. The mango leaves contain high amount of phenolic compounds such as benzoic acid, p-hydroxy benzoic acid, pyrogallol, vanillic acid, syringic acid, ferulic acid, Gallo catechin, protocatechuic acid, manindicin A&B [(6).]

Uses of Mangifera indica

Mangifera being a polyphenolic antioxidant and a glucosyl xanthone, has a strong antioxidant, anti-lipid peroxidation, immunoglobulin, cardiotonic, hypotensive, wound healing, anti-degenerative and anti-diabetic activity, denitrifies, antiseptic, astringent, diaphoretic, stomachic, vermifuge, tonic, laxative and diuretic and to treat diarrhoea, dysentery, anaemia, asthma, bronchitis, cough, hypertension, insomnia, rheumatism, toothache, leucorrhoea, haemorrhage and piles[(7)]. Phenolic compounds have the capability to quench lipid peroxidation, prevent DNA oxidative damage, scavenge free radicals and prevent inhibition of cell communications of all which are precursors to degenerative diseases. Free radicals cause depletion of the immune system anti-oxidant, change in gene

expression, induce abnormal protein resulting in degenerative diseases and ageing. Use of pure isolated compounds has been found to be less effective than the use of crude mixtures from the particular mango leaves suggesting that polyphenols are important for maximum antioxidant activity [8].

Anti-oxidant activity of Mangifera indica

The antioxidant activity of the fractions and isolated compounds from the leaves was studied using DPPH radical scavenging assay ethanolic extract. The fraction of ethyl acetate and n-butanol of mango leaves exhibited pronounced anti-oxidant activities, as they were subjected to isolation and purification of flavonoids in mango leaves. Most flavonoids have antioxidant activity based on analysis of structure activity relationship of flavonoids, the presence of catechol substitution in structure possessing a strong antioxidant activity and induce damage to biological molecule, including proteins, lipids and DNA with the activity are beta -carotene, vitamin-c and vitamin -E. The antioxidant is used for both chemo prevention and treatment purpose [9].

2. Materials and Methods

Chemicals

Ethanol, Methanol, 10% NaOH, Dil. HCL, Benedict's reagent, Fehling's reagent, Iodine, Biuret reagent, Chloroform, Alpha-glucosidase, PNPG [para nitrophenyl-alpha-glucopyranoside], Phosphate buffer, Bovine albumin serum, Folin-Ciocalteu reagent, Sodium carbonate, Gallic acid, Potassium acetate, Aluminium chloride, Rutin, 2,2-Diphenyl-2-hydrazyl (DPPH), Ascorbic acid, Sodium acetate, Glacial acetic acid, 2,3,5-triphenyltetrazolium chloride (TPTZ), Hydrochloric acid and Ferric chloride.

Collection & Authentication

The leaves of the Mangifera indica collected from nearby places of Tirupati, Andhra Pradesh and the plant specimen was authenticated by Dr. K. Madhava Chetty, Assistant professor. Botanical survey of medicinal plant unit, Sri Venkateswara University, Tirupati, AP.

Fig.2: Collection of mango leaves

Fig.3: Leaves were cut pieces and kept for drying under shade

Preparation of ethanolic extract

The plant materials were collected, washed and dried under shade. After complete drying the plant materials were powdered in a mixer to obtain a coarse powder and then passed through a 60mesh sieve and stored in an airtight container. The shade dried powdered is used for extraction. A weighed quantity of air-dried powdered drug of Mangifera indica are taken and kept for maceration respectively by ethanolic extraction. The extracts were evaporated to dryness in a rotary flash evaporator at a temperature not exceeding 60^{o}C. Phytochemical tests were carried out by following standard procedures.

Phytochemical analysis:

Chemical tests were carried out on the plant extract using standard procedures to identify the constituent molecules as described by sofowora and co-workers. Ethanolic extract of Mangifera indica are prepared. Then phytochemical analysis was carried out on plant extract.

Total flavonoid content

Total flavonoid content of the sample was determined by using methods described by faud, A. Flwi, A., and zulkifly, with slight modifications. A stock rutin standard solution of 10 mg/ml was prepared. 10mcg/ml, 20mcg/ml, 30mcg/ml, 40 mcg/ml with different concentrations of sample extracts. 1ml of sample from the stock solution was mixed with 3 ml of methanol, 0.2 ml of 1 M potassium acetate and 0.2 mL of 10% aluminium chloride 5.6 mL of distilled water was added. The solution was incubated for 30 minutes at room temperature. Two hundred microlitres of sample extracts were transferred into a 96- well plate from the centrifuge tube and the absorbance was measured at 415 nm using a spectrophotometer. The result was expressed as mg of rutin equivalent per gram of sample.

Total phenol content

Total phenolic content of Mangifera sp. Leaf extracts were analysed using the folin- ciocalteu colorimetric method described by Gao et.al. (2000). About 0.02mL of 2 mg/mL extract solution was mixed with 0.2 mL folin -ciocalteu reagent and 2 mL of distilled water. After 3 minutes, 1 mL of sodium carbonate was added. The mixture was re- incubated for 20 minutes at room temperature. Then, the absorbance was measured at 765 nm using a spectrophotometer. The total phenolic content was calculated from the gallic acid standard curve. A stock gallic acid standard solution of 1 mg/mL was prepared by dissolving gallic acid in distilled water. The result was expressed as mg gallic acid equivalent per gram of extract.

Invitro antioxidant and free radical scavenging activity

DPPH free radical scavenging activity was evaluated using the method described by Blois. Various concentration of HAEMIL and ascorbic acid (standard)ranging (2-10µg/ml) were mixed with 1 ml of freshly prepared 0.3 mM DPPH ethanol solution and 2 ml of 0.1M acetate buffer. Incubated at room temperature for 30 mins and the absorbance of resulting solution were then measured calorimetrically at 517 nm. DPPH solution (1.0 ml, 0.3Mm) treated with 1 ml of ethanol, served as negative control. ascorbic acid was used as positive control under the same assay conditions. Samples were prepared in triplicates for each analysis and the mean of absorbance was obtained. Higher absorbance indicates lower free radical scavenging activity. The percentage of DPPH scavenging activity of extract was calculated from decrease in absorbance in comparison of with control by using formula.

Results and Discussion

Figure 1: Preliminary Screening

S.No	Chemical Test	Observation	Inference
Test for Carbohydrates			
1.	Molisch's test	+	Carbohydrates is present
2.	Fehling's test	-	Carbohydrates is absent
3.	Iodine test	+	Carbohydrates is present
4.	Benedict's test	+	Carbohydrates is present
Test for proteins and amino acids			
1.	Xanthoproteic test	+	Proteins is present
2.	Biuret test	+	Proteins is present
Test for Alkaloids			
1.	Dragendorff's test	+	Alkaloids is present
2.	Mayer's test	-	Alkaloids is absent
3.	Iodine test	-	Alkaloids is absent
Test for tannins			
1.	10% NaOH	-	Tannins is absent
Test for phenols			
1.	Ferric chloride's test	+	Phenols is present
2.	Potassium dichromate's test	+	Phenols is present
3.	Iodine test	-	Phenols is absent
Test for Flavonoids			
1.	Alkaline reagent test	+	Flavonoids is present
Test for saponins			
1.	Foam test	-	Saponins are absent

Table 2: Percentage of total phenolic and flavonoid content by ethanolic extract of Mangifera indica leaves

Plant extract	Total phenolic content (%)	Total Flavonoid content (%)
Ethanolic extract of Mangifera indica	1.38 ± 0.080	4.8 ± 0.55

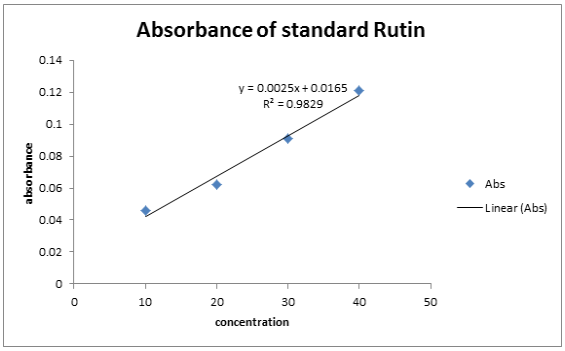

Fig 4: calibration curve of Rutin showing linearity over concentration range from 10-40µg/ml.

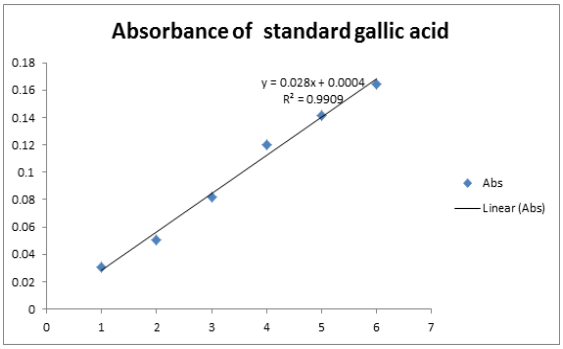

Fig.5: Calibration curve of gallic acid showing linearity over concentration range of 10-50 µg/ml.

Total Phenolic content

The total phenolic content of ethanolic extract of Mangifera indica leaves were expressed in term of gallic acid equivalent**.** The total phenolic content in the extract of M. indica leaves were 50.45%.

The total flavonoid content in samples of mangiferin sp. Leaves. The studies conducted by rutin as standard. The concentration of total flavonoid content is expressed in mg/gm of rutin equivalent. Maximum absorption depends on the concentration of flavonoids. The total flavonoid content in the extract of M. indica were 68.53 mg/gm of rutin equivalents.

In-vitro antioxidant and free radical scavenging activity of EEMIL: Radial scavenging activity of extract was observed from decrease in absorbance of DPPH with increase in concentration. Absorbance value of EEMIL had shown as 1.84, 1.98, 1.32, 0.34and 0.46 and standard ascorbic activity exhibited as 1.29, 1.12, 2.38, 3.43 and 1.85 at 2, 4, 6, 8 and 10 µg/ml respectively. EEMIL at a concentration of 2-10 µg/ml inhibited production of DPPH radical by 50-80% and showed significance scavenging effect on DPPH radical compared to standard ascorbic acid which exhibited 40-85% inhibition.

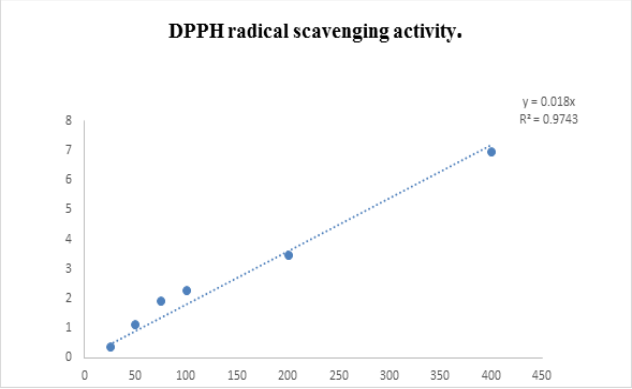

Fig.6: DPPH radical scavenging activity

Table 3: Standard values of ascorbic acid

Ascorbic acid(standard) concentration	Mean ± S. D
2µg/ml	18.93 ± 1.29
4µg/ml	41.02 ± 1.01
6µg/ml	62.8 ± 2.39
8µg/ml	68.7 ± 3.45
10µg/ml	75.30 ± 1.85

Table 4: Effect of Mangifera indica on DPPH

Concentration of extract	Mean ± S. D
20µg/ml	73.20 ± 1.85
40µg/ml	74.32 ± 1.98
60µg/ml	75.15 ± 1.34
80µg/ml	77.85 ± 0.38
100µg/ml	79.15 ± 0.47

Discussion

Preliminary phytochemical investigation confirmed that mango leaves are rich with several chemical constituents by using specific tests for each group of compounds. Mango leaves are good source phenols, flavonoids, alkaloids, glycosides, terpenoids but the absence of saponins, the present study reveals the presence of phenols and flavonoids are responsible for the anti-oxidant radical scavenging activity**.** This traditional plant is rich in phenols and flavonoids as the absorbance increases the concentration of phenolic compounds also increases and this result were equivalent with standard gallic acid mg/g. The concentration of total flavonoids were expressed as mg/g of rutin equivalents. Maximum absorption depends on the concentration of flavonoid content in the extract.

Conclusion

The present study reveals that ethanolic extract of Mangifera indica shows potent inhibition activity at different concentrations when compared with standard ascorbic acid and it has showed significant effect at 60µg/ml of extract with 10µg/ml. It is reveal that at lower concentration having high amount of phenols and flavonoids are inhibit effects is considered for the potent for further studies.

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Conflict of Interests

The authors declare no conflict of interest

Ethics Approval: Not applicable

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AI Tool Declaration

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

Data Availability

Data will be available on request

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